First Field Evidence On Circulation and Vertical Transmission of West Nile Virus Lineage-1a in Mosquitoes of Southern Iran

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Research

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Abstract

Background

Mosquitoes play an important role in the transmission of arboviruses including neuroinvasive West Nile virus. (WNV). Despite reports on seroepidemiological evidence and distribution of potential vectors of WNV in Iran, its transmission and dominant lineage(s) in mosquitoes of the Southern region has not been yet investigated. This study was conducted to inventory mosquitoes in Hormozgan province and explore them for WNV infection.

Methods

A total of 6785 larvae and adult mosquitoes were collected from 11 sites during 2015–2016. The mosquitoes were analyzed for WNV lineage detection by RT-PCR.

Results

This study revealed vertical transmission of Lineage 1a WNV in five groups including two groups of *Cx. pipiens* collected in winter and spring as well as three others containing *Cx. sitiens*, *Anopheles stephensi* and *Cs. longiareolata* collected in autumn from Bandar Abbas city.

Conclusion

The vertical transmission of WNV by effective vectors indicates the incursion of enzootic transmission in the region. The cross-species WNV transmission and prevalence of infected migratory birds coming from Europe and Siberia demand a comprehensive surveillance of WNV infection in hosts and vectors. However, vector-based surveillance remains the most accurate and feasible indicator to assess the impending risk of WNV in Iran. Given the serological and molecular evidences of WNV presence in humans, equines and birds in 26 provinces, the public health authorities should pay more attention to vector control measures to reduce the risk of WNV epidemics in the southern region of Iran.

1. Introduction

As a member of the genus Flavivirus (Flaviviridae), West Nile virus (WNV) belongs to the antigenic complex of the Japanese encephalitis virus. It is a single strand, sense positive RNA virus with a genome of about 11,000 bp in length which encodes three structural and seven non-structural proteins [1]. WNV has been reported from all continents except Antarctica and is spreading across tropical and temperate regions of the world. The natural transmission cycle of WNV involves different species of birds and ornithophilic *Culex* mosquitoes. The virus is isolated from several species of mosquitoes, but *Culex pipiens* s.l., *Culex univittatus*, *Culex neavei*, *Culex modestus*, *Culex perexiguus*, *Culex bitaeniorhynchus*,

Culex pseudovishnui, *Culex tritaeniorhynchus*, *Culex quinquefasciatus*, *Culex annulirostris*, *Culex antennatus* and *Culex vishnui* s.l. were reported as predominant vectors [2, 3]. The severity of WNV transmission to humans depends on the feeding behavior and abundance of infected mosquitoes as well as the extent of exposure to mosquito bites [4]. WNV is preserved in nature by vertical transmission within mosquitoes [5]. The viral disease in humans usually causes mild flu-like symptoms or may be asymptomatic. In less than 1% of cases, it may cause a neurological disease and sometimes death [6].

Phylogenetic studies have revealed the existence of five lineages of WNV. The lineage 1 is subdivided into two clades: the first clade (Lineage 1a) contains strains from different regions, and the second (Lineage 1b) includes the Australian Kunjin virus- KUNV [7]. The lineage 2 was mainly reported from South Africa and Madagascar, though, its circulation in Europe is increasingly evidenced since the beginning of the millennium. [8, 9]. The lineage 3 includes the Rabensburg virus, which was isolated from *Culex pipiens* in 1997 [10] and later from *Aedes rossicus* mosquitoes in the Czech Republic [11]. Lineage 4 is a West Nile virus (LEIVKrnd88–190 strain) isolated from *Dermacentor marginatus* tick in the Caucasus Mountains in northwestern Russia in 1998. The lineage 5 is a WNV strain isolated from India (strain 804994) [12]. However, only lineages 1, 2, and 5 of WNV have been associated with the disease epidemics [7].

Evidence of WNV circulation was documented in Iran's neighboring countries, namely Pakistan [13], Afghanistan [14], Azerbaijan [15], Turkey [16], Iraq [17] and the countries of the Persian Gulf such as Saudi Arabia [18], Qatar ([19] and the United Arab Emirates [20, 21]. In general, the prevalence of WNV serum in the Iranian equine population is estimated at 27.3% and in Hormozgan Province at about 40–60% [22]. In addition, several serological studies have reported the contamination of horses, birds, and humans with WNV in other provinces of Iran [23–28]. In 2012, the conjunctivitis virus Lineage 1b was isolated from *Aedes caspius* in Northwestern Iran [29]. In 2015, also, Lineage 2 was detected in *Cx. pipiens* from Northern Iran [30]. However, so far no specific surveillance strategy has been put in place to elucidate the WNV transmission cycle in Southern Iran. Therefore, the main aim of this study was to conduct extensive WNV surveillance of mosquitoes in Hormozgan Province to provide insight into the molecular epidemiology of the disease in the Southern region of Iran.

2. Materials And Methods

2.1 Study area

The surveillance was carried out in Bandar Abbas and Bandar Khamir counties, in the central part of Hormozgan Province. This region has sub-tropical climate. Mosquitoes were collected from 11 habitats, 3 of which were permanent in Siahoo village 75 km far to the North of Bandar Abbas City. The rest of specimens were captured in traps installed in various habitats of Hormozgan Province (Fig. 1). The study area comprises different microclimatic zones due to variations in local topography which includes mountains, slopes and plains. Accordingly, the sampling was performed so that to survey various elevations over four seasons from 2015 to 2016. In addition, extra sites were visited and sampled in the central part of the Province for wider surveillance of West Nile virus vectors. The land use of the sampling

sites was also observed in data record and analysis at three levels; "urban" (3 sites), "rural" (5 sites) and "natural" (3 sites) as in Table 1.

Table 1
Geographical coordinates and land use of sampling sites in Hormozgan Province (2015–2016)

No	County	Location	Latitude	Longitude	Altitude (m)	Land Use
1	SH ¹	Mountain	27°49'29"N	56°17'52"E	1040	Rural/Tangelo Gardens
2		Slope	27°45'23"N	56°19'55"E	632	Natural/ Tamarisk Shrub
3		Plain	27°45'12"N	56°20'5"E	620	Natural/ Palm Groves
4		Segh Vil.	27°47'12"N	56°22'44"E	772	Rural/Tangelo Gardens
5		Rezvan Vil.	27°33'09"N	56°05'18"E	300	Natural/Stagnant Pond
6		Sarkhun Vil.	27°23'46"N	56°25'5"E	77	Rural/Human Places
7	BA ¹	Ziba-shahr	27°12'9"N	56°18'36"E	21	Urban/Stream
8		Kamarbandi	27°11'42"N	56°17'37"E	36	Urban/Plant
9		Bahonar Sq.	27° 9'16"N	56°12'27"E	23	Urban/Stream
10		Hurmudar Vil.	27°19'18"N	56°19'23"E	65	Rural/Cow Stable
11	BK ¹	BK Spa	26°58'34"N	55°32'22"E	33	Rural/Stream
¹ SH = Siahoo, BA = Bandar Abbas City; BK = Bandar Khamir.						

2.2 Mosquitoes Collection

To verify the vertical transmission of WNV in Culicidae mosquitoes, larvae, male and female mosquitoes were collected and tested. Larvae from different breeding sites was collected by the standard dipper method. The larvae were identified at genus levels before being confined into collection bottles, on which breeding site characteristics, date of collection and the name of identified genus were recorded. Most larvae were transferred to the insectarium and reared using a 9% sterile sugar solution under proper temperature conditions. Adult mosquitoes were trapped by different methods including hand catch, bed net trap with human prey, smoke purging in septic tanks, Co2-Trap and BG-Trap. The sample collections, applied techniques and methodology of the present study were approved by TMU Committee for Ethics in Biomedical Research under the License No. IR.TMU.REC.1394.214 dated 2nd February 2016.

2.3 Sample Identification and Coding

The collected mosquitoes were identified using Azari-Hamidia & Harbach's Morphological Key [31] when in chill boxes. Then, they were categorized and coded separately based on the species, sampling method, date and place. Finally, cryotubes containing classified mosquitoes were transported to the laboratory in

liquid nitrogen portable tank and frozen until used for virus detection. Coding was done in such a way that the first letter of the corresponding code stands for the region (Mountain: M, Domain: D, Plain: P, Rezvan: R, Hormodar: H, Segh: S, Falake-bahonar: F, Zibashahr: Z, Bandar- khamir: B, Bandar-Abbas Belt Street: T and Sarkhun: K), the following figure indicates the season (1 = autumn, 2 = winter, 3 = spring, 4 = summer), the next two letters indicates the sampling methods (DO = dipper method, Di = larvae caught by dipper method and reared in the insectarium, BN = Bed Net Trap, HC = Hand Catch, Sm = smoke purging into septic tank, CO = Co2- Trap and BG = BG-Trap) and the last figure stands for the species (1: *An. stephensi*, 2: *Culex sitiens*, 3: *Cx pipiens*, 4: *Cx theileri*, 5: *Cx perexiguus*, 6: *Culiseta longiareolata*, 7: *Aedes caspius*, 8: *An. sergenti*, 9: *An. dthali* and 10: *An. fluviatilis*). For example the code K2HC3 represents the adult of *Cx. pipiens* mosquitoes collected during the winter season by manual catch from Sarkhoon village. The mosquito specimens were deposited in the Entomological Archives of the Department of Parasitology and Medical Entomology of Tarbiat Modares University under No. HoPEB-1395.

2.4 RNA Extraction and RT-PCR

In this study, 80 groups of mosquitoes, each including 1 to 100 specimens, were pooled (each pool $\leq$ 20 specimens) and tested for five different WNV lines using degenerate primer and RT-PCR technique. First, the samples were homogenized by MicroPestle in RNase & DNase Free microtubes (1.5 ml) in 145 μ l DEPC treated water. RNA was then extracted using QUIAGEN Viral RNA Extraction Kit (Cialog Viral RNA Kit- Catalog no.: 52904). The cDNA was generated using the GenAll kit (HyperScriptTM First strand synthesis kit- Cat.no. 601-005). Both molecular analyses were performed according to the manufacturer's protocol. The resulting cDNA was used for PCR analysis.

The degenerate primer was designed based on the previously reported primer of the prM-envelope region (nt 233–640) of the five-lineages sequences of WNV. The primer sequences used were: Forward 5'-TTGTGTTGGCTCTCTTGGCGTTCTT-3' (nt 212 to 236) and reverse 5'-CAGCCGACAGCACTGGACATTCATA-3' (nt 619 to 643). All samples were re-tested using a second RT-PCR, with the degenerate primer. In addition, to make cDNA, PCR was used with the specific primer of the WNV gene.

For all PCR reactions, the bacterium containing plasmid coding prM – envelope region of West Nile virus Lineage 1 and distilled water were used as positive and negative controls respectively. The plasmid was synthesized by General Biosystems Co. in pUC57 vector and cloned in E. coli TOP10 bacterium. The cloning and addition of the bacterium containing WNV plasmid to the positive control tube were done in a separate room and airflow cabinet to avoid contamination. The temperature profile of PCR was as follows: 1 cycle 95 °C for 4 min. (Initial denaturation) and then 35 cycles [95 °C for 1 min. (Denaturation), 58 °C for 1 min. (Annealing) and 72 °C for 1 min. (Extension)] and 1 cycle at 72 °C 10 min. (Final extension). Finally, PCR products were analyzed by 1.5% (w/v) agarose gel electrophoresis. PCR products of the expected size (408 bp) were scored as positive.

2.5 Phylogenetic analysis

The corresponding band (408 bp) was purified before being sequenced by South Korea's Macrogen Company. The obtained results of sequencing were examined using BLAST

(<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The WNV strains were then retrieved from the public database (<http://www.ncbi.nlm.nih.gov/genbank/>) based on their host and the geographical origin of detection for the 5-lineages of WNV. Phylogenetic analysis was done using a total of 30 sequences of WNV strains including those from GenBank, as well as the WNV sequences obtained in this study. The sequence of Japanese encephalitis virus – JEV (accession no. U04522.1) was used as an out group. The nucleotide sequences were aligned using the Clustal W DNA algorithm. Then, the phylogenetic tree was constructed using the Maximum Likelihood method based on JTT + I + G model [32]. The evolutionary analysis was conducted in Mega7 software [33]. To assess the robustness of the phylogenetic tree, bootstrapping was computed with 1000 repetitions.

3. Results

3.1 Identification of collected mosquitoes:

In this study, a total of 6785 mosquitoes were collected and identified, of which 1592 individuals were screened for WNV-RNA by RT-PCR technique (Table 2). The mosquitoes were classified in four genera namely *Anopheles*, *Culex*, *Culex* and *Aedes* and in ten species including *An. stephensi*, *An. dthali*, *An. fluviatilis*, *An. sergenti*, *Cx. pipiens*, *Cx. theileri*, *Cx. sitiens*, *Cx. prexiguus*, *Cs. longiareolata* and *Ae. caspius*.

Table 2

Mosquitoes sample codes and their corresponding sample size collected and analyzed (pooled) by RT-PCR in Hormozgan Province

Site	Sample codes	Respective sample size (pooled)
Plain	*P1Di1, *P1Di2, *P1Di6, P1Di7, P2BN1, P2BN3, P2BN7, P2Di1, P2Di6, P2Di7, P3BN7, P3Di1, P3Di3, P3Di7, P3Di9, P3DO1, P3DO3, P3DO7, P4BG1, P4BG3, P4BG7, P4BG9, P4CO1, P4CO1, P4CO2, P4CO3, P4CO7, P4CO8, P4CO9	*19 (19), *16 (16), *12 (12), 84 (84), 1 (1), 14 (14), 7 (7), 16 (16), 46 (46), 6 (6), 8 (8), 10 (10), 1 (1), 1 (1), 1 (1), 81 (81), 35 (35), 32 (32), 5 (5), 4 (4), 9 (9), 7 (7), 3 (3), 1 (1), 1 (1), 13 (13), 100 (100), 1 (1), 1 (1)
Slope	D1Di1, D1Di4, D1Di5, D1Sm3, D2Sm3, D3Di1, D3Di3, D3Di7, D3DO1, D3DO3, D3DO7, D4BG7, D4CO1, D4CO10, D4CO3, D4CO7, D4CO3	7 (7), 4 (4), 2 (2), 3400 (100), 921 (100), 2 (2), 13 (13), 25 (25), 10 (10), 38 (38), 90 (90), 1 (1), 7 (7), 3 (3), 34 (34), 14 (14), 34 (34)
Mountain	M1Di1, M1Di2, M1Di3, M2Di1, M2Di3, M2Di6, M2Di7, M3Di3, M3Di6, M3DO3, M3DO6, M4BG9, M4CO1, M4CO3, M4CO7, M4CO8	12 (12), 5 (5), 5 (5), 1 (1), 1 (1), 1 (1), 7 (7), 22 (22), 2 (2), 73 (73), 8 (8), 1 (1), 3 (3), 12 (12), 1 (1), 1 (1)
Others	B4DO1, B4DO7, *T2Sm3, T4CO3, R2BN3, R2BN5, R2BN6, R2Di6, Z2Di3, Z2Di6, F3DO3, S3BN3, S3DO1, *S3DO3, H2Di4, H2Di8, H2DO6, K2HC3	40 (40), 5 (5), *40 (40), 3 (3), 20 (20), 1 (1), 1 (1), 7 (7), 947 (100), 325 (100), 5 (5), 2 (2), 11 (11), 26 (26), 1 (1), 1 (1), 40 (40), 15 (15)
* WNV-positive mosquito samples.		

3.2. Screening for WNV infection

The RT-PCR analysis confirmed the presence of West Nile virus in five pooled samples categorized under the codes P1Di1, P1Di2, P1Di6, S3DO3 and T2Sm3. These samples included 19, 16, 12, 26, and 40 mosquitoes respectively. The WNV isolates were subjected to subsequent sequencing. The sequence data of the WNV isolates were deposited in GenBank under accession numbers: MW455335, MW455336, MW455337, MW455338 and MW455339.

Three species of mosquitoes captured from Siahoo Village of Bandar Abbas County in autumn were found infected with West Nile virus. These species were *An. stephensi*, *Cx. sitiens* and *Cs. longiareolata*. West Nile virus was also present in *Cx. pipiens* caught from Sagh village on the outskirts of Bandar Abbas city in spring, whereas the same infected species were trapped from Kamarbandi Street inside Bandar Abbas city during the winter season.

3.3 WNV transmission

Our study confirmed the vertical transmission of WNV in *Cx. pipiens*, *Cx. sitiens*, *An. stephensi* and *Cs. Longiareolata*. In fact, the viral infection was detected in these species as members of larval samples coded P1Di1, P1Di2, P1Di6, which were reared to adult stages under insectary conditions. Also, the virus was isolated from the same species either as members of larval samples coded S3DO3 or adult females

and males from samples coded T2Sm3. The tested mosquitoes varied in their ability to vertically transmit WNV (Table 3). The overall MFIR was ≈ 3.1 infected adult progeny per 1,000 tested.

Table 3
Summary of West Nile Virus Vertical Transmission by identified mosquito species

Species	Larva	Adult	Total	PP/TL ¹	PP/TA ¹	% VT	MFIR ¹
<i>Cx. sitiens</i>	21	1	22	1/2	0/1	50	45/1000
<i>Cx. pipiens</i> s.l.	142	568	710	0/6	2/18	11	2.8/1000
<i>Cx. theileri</i>	4	1	5	0/1	0/1	0	0/1000
<i>Cx. perexiguus</i>	2	1	3	0/1	0/1	0	0/1000
<i>Cs. longiareolata</i>	168	49	217	1/6	0/3	16.6	4.6/1000
<i>Ae. caspius</i>	123	267	390	0/5	0/10	0	0/1000
<i>An. sergenti</i>	0	3	3	0/0	0/3	0	0/1000
<i>An. dthali</i>	1	9	10	0/1	0/3	0	0/1000
<i>An. fluviatilis</i>	0	4	4	0/0	0/2	0	0/1000
<i>An. stephensi</i>	67	161	228	1/7	0/9	14.3	4.3/1000
Total	528	1064	1585	3/29	2/51	6.2)	3.1/1000

¹ P/TL = positive pools/tested larvae; PP/TA = positive pools/tested adults; MFIR = Minimum number of mosquitoes infected with West Nile virus per 1,000 offspring)

3.4. Analysis of virus sequences

Molecular Phylogenetic analysis revealed that the five sequences of WNV obtained from positive samples in this study exhibited high similarity with WNV Lineage 1a strains isolated from Russia, Europe, the Middle East, Africa, and the United States. Also, as shown in Fig. 2 and Additional Figure S1, the isolated strains were more closely related since they clustered together in one branch.

4. Discussion

Despite the nationwide serological evidence on the prevalence of WNV since 1976 [27] and the establishment of Arbovirus monitoring system since 2000, there is a paucity of information about WNV vectors in Iran [29]. Also, the distribution map of the globally spreading Lineage 1a of West Nile Virus as a serious public health problem still not clear at national level [34]. The present study aimed to contribute towards WNV vectors identification and surveillance. This information can help to anticipate new

endemic foci, monitor transmission dynamics and identify different mosquito vectors that could transmit the virus to animal hosts, reservoirs and people.

Our results confirmed the vast geographical distribution of Lineage 1a of WNV in mosquito vectors. For the first time, this study reported the infection of four Culicidae species namely *Cx. pipiens*, *Cx. sitiens*, *An. stephensi* and *Cs. longiareolata* with Lineage 1a WNV strains in Bandar Abbas County including the provincial capital in winter and spring seasons. Hormozgan Province had been previously designated to accommodate WNV seropositive horses [22]. Ziyaeyan et al reported the circulation of WNV in people and mosquitoes in Hormozgan Province [35].

In agreement with reports on mosquito species which were found infected with WNV, we detected the virus in three mosquito genera namely *Culex*, *Culiseta* and *Anopheles* [35, 36]. It is stated that the main transmission cycle of WNV in nature involves *Culex* spp mosquitoes and birds. The species *Cx. pipiens*, is also known to be the main vector of the virus and responsible for its both enzootic and epizootic transmission cycles in urban areas of the United States [36], Europe and Russia [37, 38]. But the species *Cx. sitiens* can play a regional and complementary role in the transmission [36]. The species *Cx. sitiens* was introduced as the new WNV vector in India [39]. Although the species has shown relatively weak vectorial capacity for transmitting WNV NY-99 in laboratory, it is still important for being ornithophilic and prevalent in coastal areas. It can also build up its population very fast under favorable conditions [40]. The species *Cs. longiareolata* is also known to transmit WNV and feed predominantly on birds and rarely on humans [39]. However, so far there has been no report on *An. stephensi* involvement in WNV transmission to humans. Nonetheless, as an anthropophilic mosquito, the anopheline may contribute to the disease outbreak in seasons coinciding with its high population and peak activity when infected birds become its immediate feeding sources.

This study demonstrated the presence of WNV in *Cx. pipiens* in Bandar Abbas City and the neighboring Sagh Village as a primary health concern, In addition the viral infection of *Cx. sitiens* and *Cs. longiareolata* in Siahoo village and the surrounding plain areas was shown as a secondary hazard. More importantly, the present study reports for the first time the vertical transmission of WNV Lineage 1a in *Cx. sitiens*, *An. stephensi* and *Cs. longiareolata*, though the vertical transmission of the virus has been already shown in 14 mosquito species, including *Cx. pipiens* [41–50]. The vertical transmission of WNV through ornithophilic mosquitoes may not only stabilize the endemicity of the disease in Hormozgan Province, but also complicate its epidemiology and control measures.

The minimum filial infection rate (MFIR) obtained in the current study for *Cx. pipiens* s.l. is 2.8/1000, exactly similar to that reported by Anderson et al. [46]. Other researchers have obtained MFIR values for *Cx. pipiens* s.l., which were between 0.52/1,000 and 4/1,000 [44, 47, 51]. The filial infection rate of 4.3/1000 in *An. stephensi* and 4.6/1000 in *Cs. Longiareolata*. are comparable to those stated by Baqar et al. [45]. In our study, *Cx. sitiens* from Siahoo village has shown the highest MFIR equal to $\approx 45/1,000$, since 1 of 3 pools was found infected with detectable virus. The discrepancy in vertical transmission rates between *Cx. sitiens* and other species may be attributed to its higher susceptibility to WNV infection

as well as the small sample size collected and tested compared to other species. The overall MFIR value of WNV infected mosquito species in Hormozgan Province was equal to 3.1 infected progeny per 1000 individuals. This value is similar to both field and laboratory results reported by other researchers for *Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. tarsalis* [44, 47, 52]. Our results emphasize the findings that vertical transmission rates depend on various combinations of vector mosquito species, viral strains, and environmental conditions [53]. Over the study area, which included different biotopes, the infected mosquitoes were detected only in autumn. On the other hand, except for *Cx. sitiens*, the vertical infection rates of field collected mosquitoes were less than 20–30 % which hardly satisfy the least required rates for viral persistence in nature [54]. These evidences may indicate the importance of re-introduction of viral load by migratory birds in addition to vertical transmission of WNV to maintain the virus in Hormozgan Province. Both vertical transmission and re-introduction of WNV in the region not only support the detectable virus in autumn but also the virus persistence in overwintering female mosquitoes. The effective vertical transmission of WNV by ornithophilic mosquitoes corroborates the endemicity of WNV and complicates intervention measures in Hormozgan Province.

In plain sites of Siahoo Village, three mosquito species were found infected with the virus, whereas no infection was detected in mosquitoes trapped in slopes and mountainous areas. This can be attributed to more favorable climate conditions, and richer wildlife and vegetation diversity in Siahoo plain compared to elevations. In addition, WNV was isolated from mosquitoes of Bandar Abbas City in three consecutive seasons of autumn, winter and spring during 2015–2016 but not in summer. This may be due to the fact that every year from autumn to spring, Hormozgan Province provides wetland and coastal refuges for migratory bird flocks arriving from cold regions of Siberia and Europe, a number of which harbor and transmit WNV to local ornithophilic mosquitoes [55].

Based on phylogenetic analysis, only West Nile virus Lineage 1a was detected in infected mosquitoes of Bandar Abbas County in Hormozgan Province, while Lineage 1b strains were reported from *Aedes caspius* mosquitoes and from a patient with encephalitis in northwestern and central Iran respectively. [29, 56] However, the WNV isolated from *Cx. pipiens* in northern Iran was shown to be a Lineage 2 isolate. So far, there is no comprehensive map indicative of geographical distribution and epidemiological patterns of dominance for various lineages of West Nile virus in Iran. In fact, a nationwide surveillance is still lacking to determine the phylogenetic status of native and exotic WNV strains in the country. However, the involvement of birds especially the migratory ones as amplifying hosts and various mosquitoes as vectors in the natural cycling of WNV add more complications to distribution and transmission dynamics of the viral strains. This is particularly true given the locomotion of the components of epidemiologic triangle of WNV under rapidly changing global environment [57, 58]. Therefore, the phylogenetic variation of WNV strains at national level appears to be plausible. The presence of migratory birds harboring WNV from their origin in Europe and Russia in Hormozgan wetlands and coastal areas over 3 seasons of the year may stand behind the similarity of the strain isolated in this study with those reported from Russia, Italy and Spain. By the same token, the same argument can be put forward for the presence of WNV Lineage 1a in Dubai, United Arab Emirates [21], for their neighborhood to Hormozgan Province and shared commonality. (Fig. 2)

West Nile Lineage 1a is the most serious strain for its association with encephalitis epidemics as well as epizootics of high mortality to birds and horses in many parts of the worlds. [59] Although, no WNV outbreak has been reported in Iran, the serological studies have already confirmed its presence in humans, equines and birds in 26 provinces of Iran. [60]. Therefore, public health officials in Iran should pay more attention to vector control measures to reduce the risk of WNV epidemics in southern region of Iran.

Abbreviations

NV

West Nile virus; RT-PCR:Reverse transcription polymerase chain reaction; MFIR:Minimum Filial Infection Rate;

Declarations

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Availability of Data and Materials:

All data generated or analysed during this study are included in this published article and its supplementary information file (Figure S1).

Author Contributions: *Conceptualization*, MSD & SHMK; *Data curation*, EBI; *Analysis*, EBI & MSD; *Funding acquisition*, MSD; *Investigation*, EBI & SHMK; *Methodology*, MSD & SHMK; *Project administration*, MSD; *Software*, EBI; *Supervision*, MSD & SHMK; *Writing – original draft*, EBI; *Writing – review & editing*, MSD & SHMK.

Ethics Approval and consent to participate: The sample collections, applied techniques and methodology of the present study were approved by TMU Committee for Ethics in Biomedical Research under the License No. IR.TMU.REC.1394.214 dated 2nd February 2016.

Competing Interest: The authors declare no conflict of interest.

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Figures

Figure 1

Geographic location of study area: 1=Mountain; 2=Domain; 3=Plain; 4=Segh village; (1 to 4 are in Siahoo); 5=Rezvan village; 6=Sarkhun village; 7= Ziba-shahr District (Bandar-Abbas); 8= Bandar-Abbas Kamarbandi; 9= Bahonar Squ.(Bandar-Abbas); 10= Hormodar village; 11= Bandar-Khamir Spa.

Figure 2

Phylogenetic tree based on a 408-nt sequence (nt 233–640) of 30 strains of West Nile virus (WNV) constructed using the Maximum Likelihood method. The evolutionary distances were computed based on the JTT+ I + G model. The Japanese encephalitis virus (JEV) was used as an out group. Bootstrap percentages are shown at the tree nodes. GenBank accession numbers, host (in parentheses), Location of virus isolation and date collection are provided.

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